

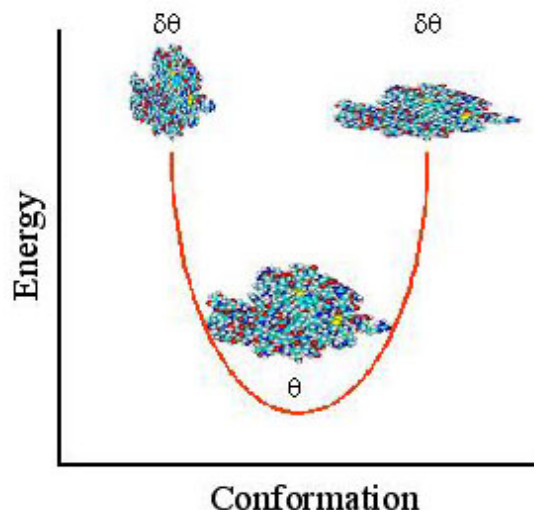
Quantitative Optical Spectroscopy of Biologicals

Biopharmaceuticals, protein arrays, proteins for research purposes, and protein standards must have well-characterized molecular compositions and conformations to allow comparisons of treatment protocols, disease diagnosis, etc. In collaboration with the FDA and the New York Academy of Sciences, NIST hosted a meeting, in December of 2005, entitled "Follow-on Biologicals: Scientific Issues in Assessing the Similarity of Follow-on Protein Products" to discuss the measurements that can be used to characterize proteins. A particular theme of this meeting was to identify techniques that can distinguish between measuring molecular averages and the distribution of properties such as glycoform and conformations.

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NIST is developing a method to obtain more information about molecular conformational distributions using spectroscopy. This will allow us to distinguish between an ensemble of molecules with only a limited number of molecular conformations and an ensemble of molecules with an extended distribution of conformations that happen to have the same average values.

In 2005, we utilized internal reflection infrared spectroscopy measurements of self-assembled alkanethiol monolayers tethered to gold surfaces to validate our method. We form a P_2 orientation order parameter that depends not only on an average orientation angle, θ , but also on the deviation from that average, i.e., the width of the orientation distribution, $\delta\theta$. Comparisons of the parameter P_2 , obtained from the analysis of internal reflection optical measurements on self-assembled alkanethiol monolayers tethered to gold surfaces, with parameters derived from a molecular dynamics simulation of the same system show good agreement. Further comparisons of our new parameters between samples with different degrees of order suggest that we are measuring conformational heterogeneity in alkanethiol monolayers. This seems reasonable based on the idea that there can be no single molecular orientation when dealing with flexible molecules, i.e., each molecule has its own orientation as seen in the figure.



NIST researchers have made measurements of $\delta\theta$ as a function of the extent of deuterium exchange for hydrogen in isotropic films of proteins. Our preliminary results indicate that our orientation distribution work can be generalized to protein conformational heterogeneity in isotropic samples.

Future Plans: In FY06, we will apply our monolayer work to more accurate quantitation of proteins on surfaces and to continued efforts at measuring protein structural heterogeneity in isotropic samples.